

小菜蛾有机磷抗性季节性变化及毒理机制研究

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摘要: 1998~2003 年期间的田间监测结果显示, 福州地区小菜蛾 *Plutella xylostella* 对甲胺磷和敌敌畏的抗性水平呈季节性变化, 每年春秋两季的抗性水平显著高于夏季 7 月期间的抗性水平。与 25 °C 2d 的饲养条件相比, 35 °C 2d 和 5 °C 30d 可使小菜蛾羧酸酯酶 (CarE) 和谷胱甘肽-S-转移酶 (GST) 活力显著抑制, 35 °C 2d 还可使小菜蛾乙酰胆碱酯酶 (AChE) 活力显著抑制, 但 5 °C 4d 对小菜蛾 AChE、CarE 和 GST 活力影响差异不显著。当反应温度均为 25 °C 时, 在 35 °C 2d、25 °C 2d 和 5 °C 30d 下饲养的小菜蛾 AChE 对甲胺磷和克百威的敏感性 (K_i 值) 没有明显变化, 但酶与杀虫剂在 37 °C 的反应温度下, 甲胺磷、敌敌畏和克百威对在 25 °C 下饲养的小菜蛾 AChE 的抑制作用显著增高。上述结果表明环境温度会影响小菜蛾的解毒代谢及杀虫剂对 AChE 的抑制作用。探讨了环境温度的季节性变化对田间小菜蛾有机磷抗性水平影响的毒理学机制。

关键词: 小菜蛾; 有机磷抗性; 环境温度; 乙酰胆碱酯酶敏感性; 解毒酶

Seasonal dynamics of the resistance to organophosphorus insecticides and its biochemical mechanism in *Plutella xylostella* (L.)

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Abstract: The resistance to methamidophos and dichlorvos in the field populations of *Plutella xylostella* (Lepidoptera: Yponomeutidae), collected from Fuzhou, Fujian, China from October 1998 to January 2003, was determined using a leaf-dipping method with 3rd instar larvae. *P. xylostella*'s resistance to insecticides, methamidophos and dichlorvos, was significantly higher in autumn and spring than in summer. Apparently, seasonal variations of the insecticide resistance existed in the field population of *Plutella xylostella*. Therefore, the effects of temperature on the activities of acetylcholinesterase (AChE), carboxyesterase (CarE) and glutathione-S-transferase (GST), and the bimolecular rate constant (K_i) of AChE in the larvae of *P. xylostella* were investigated.

The AChE activity was determined with acetylthiocholine (ATCh) iodide as a substrate in the presence of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) in the pH 7.8 phosphate buffer at 25 °C. The CarE activity was measured with 1-naphthyl acetate (α -NA) as a substrate in the pH 7.8 phosphate buffer containing eserine at 37 °C. The GST activity was measured with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate in the pH 7.4 phosphate buffer at 25 °C. To determine the K_i of AChE, AChE solutions were mixed with insecticides and incubated at various constant temperatures in a water bath. At different time periods, the incubated solutions were sampled for residual enzymatic activities in an ATCh solution. All experiments were carried out in triplicate, in the presence or absence of the insecticides.

In order to study the temperature effects of AChE, CarE and GST on the enzymatic activities, *P. xylostella* larvae were reared in an environmental chamber at 35 °C and 25 °C for 2 days, and at 5 °C for 4 and 30 days. Then, the 4th instar larva was used for the determination of AChE, CarE and GST activities. The biochemical analysis for the larvae with the different

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rearing temperatures was conducted simultaneously. In comparison with the larvae reared at 25 °C for 2 days, the activities of CarE and GST were significantly inhibited when reared at 35 °C for 2 days or 5 °C for 30 days. The AChE activity was also considerably inhibited when reared at 35 °C for 2 days. There was no apparent activity inhibition on the three enzymes observed when the *P. xylostella* larvae were reared at 5 °C for 4 days.

P. xylostella larvae were also reared in an environmental chamber at 35 °C and 25 °C for 2 days and 5 °C for 30 days to study the rearing temperature on the *K_i* value of AChE. The 4th instar larva was subsequently used for the *K_i* determination. The enzyme and insecticide mixtures were incubated at 25 °C. No significant effect on the *K_i* of AChE to the methamidophos and carbofuran in *P. xylostella* larvae was found.

Under the constant 25 °C rearing temperature, the incubating temperature was found to significantly affect the *K_i* of AChE of *P. xylostella* larvae to the insecticides. The *K_i* values to methamidophos, dichlorvos and carbofuran at 37 °C were 1.33, 1.60 and 1.75 times higher than those incubated at 25 °C, respectively.

The results suggested that the environmental temperature could affect the detoxifying metabolism and the inhibition of AChE in *P. xylostella*. The effects of the seasonal variation of temperature on the resistance to organophosphates and its toxicological mechanism in *P. xylostella* were discussed.

Key words: *Plutella xylostella*; organophosphates resistance; environmental temperature; acetylcholinesterase sensitivity; detoxification enzymes

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由于杀虫剂的长期大量使用,已导致福州地区小菜蛾 *Plutella xylostella* (L.)对生产上常用的各类杀虫剂产生不同程度的抗药性^[1],小菜蛾对甲胺磷的抗性机制与乙酰胆碱酯酶敏感性降低及解毒酶活性增高有关^[2~4]。昆虫在不同测试温度下对杀虫剂的敏感性差异显著,这已为许多研究所证实,有机磷对昆虫的毒性与测试温度呈正相关关系^[5]、而拟除虫菊酯类杀虫剂对昆虫的毒性则随不同种昆虫表现为毒性与测试温度呈正相关关系或负相关关系^[6]。杀虫剂选择压力将导致昆虫抗性的发展,这已为人们所共识,但国际上有关气候因素对昆虫抗性影响的研究尚少,已有报道低温可抑制小菜蛾羧酸酯酶(CarE)活力^[7,8],夏季与冬季抗马拉硫磷小菜蛾幼虫对马拉硫磷的抗性水平差异巨大^[8]。作者在 1998~2002 年期间对小菜蛾抗性监测中发现,在相同的测试温度(25 °C)下进行杀虫剂的生物测定,一年间小菜蛾对有机磷的抗性水平呈春秋季节较高、夏季较低的季节性变化特点,在此基础上进行了毒理机制的研究,结果报道如下。

1 材料与方法

1.1 供试昆虫与毒力测定

从福州建新蔬菜基地田间采集的抗性小菜蛾用无农药甘蓝菜幼苗在 25 °C 下、L:D=16:8 条件下饲养,取 F1 代 3 龄幼虫采用叶片浸渍饲喂法,在 25 °C、L:D=16:8 下进行杀虫剂毒力生物测定^[9],48 h 后检查死虫数,计算 *LC*₅₀。

1.2 化学试剂和供试杀虫剂

1-氯-2,4-二硝基苯(CDNB)、还原型谷胱甘肽(GSH)及碘化硫代乙酰胆碱(ATCh)(均为 Sigma 产品);5,5-二硫-双(2-硝基苯甲酸)(DTNB)(Carl Roth 产品);毒扁豆碱(Fluka 产品); α -乙酸萘酯(上海试剂一厂产品);其它药品或试剂(国产分析纯或化学纯)。90%甲胺磷原油(三明农药厂);92.5%敌敌畏原油(湖北沙隆达股份有限公司);98%克百威原药(福安农药厂)。上述药剂中的甲胺磷和克百威属菜田中不能使用的高毒杀虫剂,本文仅用作室内毒理研究。

1.3 乙酰胆碱酯酶(AChE)、谷胱甘肽-S-转移酶(GST)及羧酸酯酶(CarE)活性测定

将田间抗性小菜蛾 F1 代 4 龄幼虫于 1/15 pH 8.0(用于 AChE)、pH 7.4(用于 GST)或 pH 7.0(用于 CarE)的磷酸缓冲液中冰浴匀浆,于 0 °C、4000r/min(用于 AChE 和 CarE)或 10 000g(用于 GST)下离心,取上清液作酶源。参照 Ellman 等^[10]的方法测定 AChE 活性,参照吴刚等(2001)的方法测定 CarE 和 GST 活性^[10]。蛋白质浓度测定根据 Bradford^[11]方法进行。

1.4 双分子速度常数(*K_i*)测定

将酶液与抑制剂在 25 °C(用于 AChE)下保温,在一定的时间间隔内取一定量的保温混合液加入到反应系统中,测定剩余酶活力,按照 Aldridge^[12]的方法计算 *K_i* 值。AChE 的反应温度分别为 25 °C。

1.5 环境温度对小菜蛾 AChE、CarE 和 GST 活性的影响

取原饲养于 25 °C 的田间抗性小菜蛾 2~3 龄幼虫置于 5 °C 人工气候箱中,30d 后取发育至 4 龄的幼虫用于酶活性测定。取原饲养于 25 °C 的田间抗性小菜蛾在人工气候箱中分别于 35 °C 2d、25 °C 2d 和 5 °C 4d 饲养后,取 4 龄幼虫用于酶活性测定,3 种饲养温度对酶活性影响的生化测定同时进行。

1.6 不同温度下饲养的抗性小菜蛾 AChE 的 K_i 值测定

取原饲养于 25℃ 的田间抗性小菜蛾分别在人工气候箱中于 35℃ 2d、25℃ 2d 和 5℃ 30d 下饲养,取 4 龄小菜蛾幼虫,按 1.4 的方法,在 25℃ 下测定 AChE 的 K_i 值。

1.7 不同反应温度下抗性小菜蛾 AChE 的 K_i 值测定

取人工气候箱中于 25℃ 下饲养的 4 龄初田间抗性小菜蛾幼虫制备酶液,将酶与抑制剂的混合液分别在 37℃、25℃ 和 5℃ 下保温,在一定的时间间隔内取一定量的保温混合液加入到反应系统中,按 1.4 的方法分别于 37℃、25℃ 和 5℃ 的反应温度下测定 K_i 值。

2 结果与分析

2.1 田间小菜蛾的抗药性监测

1998~2003 年福州地区小菜蛾对甲胺磷和敌敌畏的抗性水平的田间监测结果列于表 1,1998 年 10 月和 11 月对甲胺磷和敌敌畏的抗性水平最高,之后,抗性水平明显下降。一年间抗性变动规律显示,每年春秋季节抗性水平较高,而夏季 7 月期间抗性水平最低,且与其它时期的抗性水平相比差异巨大。

表 1 1998~2003 年期间福州田间小菜蛾幼虫甲胺磷和敌敌畏抗性消长动态

Table 1 Seasonal dynamics of insecticide resistance in the field populations of <i>P. xylostella</i> larvae during from 1998 to 2003 in Fuzhou				
年-月 Year-Month	甲胺磷 Methamidophos		敌敌畏 Dichlorvos	
	LC_{50} (95%CL) mg/L	Slope±SE	LC_{50} (95%CL) mg/L	Slope±SE
1998-10	3154(2883~3450)	2.22±0.35	1470(1237~747)	2.33±0.42
1998-11	2750(2479~3050)	2.23±0.26	1023(835~1253)	2.15±0.49
1999-07	471(415~535)	2.72±0.49	310(237~404)	1.52±0.11
1999-10	2121(1781~2527)	2.60±0.24	1864(1444~2407)	1.65±0.31
1999-12	2038(1859~2235)	2.78±0.32	1623(1378~1911)	2.72±0.31
2000-04	1327(1156~1525)	2.15±0.56	1307(1082~1578)	2.29±0.60
2000-07	542(492~597)	3.28±0.20	346(279~430)	1.94±0.27
2000-11	1758(1491~2070)	1.62±0.37	1086(867~1361)	1.88±0.26
2001-04	1619(1477~1776)	2.53±0.73	801(735~873)	2.68±0.47
2001-07	782(730~838)	2.63±0.20	265(219~320)	2.31±0.45
2001-11	1258(1175~1345)	2.29±0.14	773(625~955)	2.01±0.47
2002-04	1780(1460~2170)	2.33±0.40	912(692~1202)	1.44±0.16
2002-07	703(584~846)	3.08±0.38	372(295~468)	1.75±0.22
2002-11	1613(1379~1885)	2.93±0.30	1008(801~1269)	1.77±0.10
2003-01	1495(1218~1839)	2.36±0.39	977(760~1258)	1.62±0.15

2.2 饲养温度对抗性小菜蛾幼虫 AChE、CarE 和 GST 活力影响的研究

与饲养于 25℃ 2d 的小菜蛾相比,短期高温(35℃、2d)和长期低温(5℃、30d)的环境温度均可导致抗性小菜蛾 CarE 和 GST 活力被抑制,且短期高温(35℃、2d)也可导致抗性小菜蛾 AChE 活力被抑制,但短期低温(5℃、4d)对抗性小菜蛾 AChE、CarE 和 GST 活力影响均不大(表 2)。

2.3 温度对抗性小菜蛾幼虫 AChE 敏感性的影响

当酶与杀虫剂反应的温度均为 25℃ 时,在 35℃ 2d、25℃ 2d 和 5℃ 30d 下饲养的抗性小菜蛾 AChE 对甲胺磷和克百威的敏感性(K_i 值)没有明显变化,表明饲养温度的改变对田间抗性小菜蛾幼虫 AChE 对杀虫剂的敏感性没有明显影响(表 3),而酶与杀虫剂保温温度对在 25℃ 下饲养的抗性小菜蛾 AChE 对甲胺磷、克百威和敌敌畏的敏感性影响显著,在 37℃ 高温下可显著增高抗性小菜蛾幼虫 AChE 对甲胺磷、克百威和敌敌畏的敏感性(表 4)。

3 讨论

田间监测结果显示福州地区小菜蛾已对生产上常用有机磷、氨基甲酸酯、拟除虫菊酯类杀虫剂、阿维菌素、锐劲特、抑太保和 Bt 产生不同程度的抗药性^[1]。温度会影响小菜蛾的适合度,当饲养温度大于 32.5℃ 时,小菜蛾生长发育受显著抑制^[13, 14],福州地区小菜蛾在 6~8 月份高温期间,田间虫口密度显著下降^[15]。此外,环境气温也会显著影响小菜蛾解毒酶 CarE 的活性^[7],Maa and Chuang 报道了温度对抗马拉硫磷小菜蛾幼虫的影响,夏天幼虫 LD₅₀为 30μg/虫,冬天为 140μg/虫,敏感性降低 4.7 倍^[8]。本文的研究证实高温可显著影响小菜蛾对有机磷的抗性机制,福州地区每年 7~8 月期间白天田间气温常高达 30~39℃,在 35℃ 饲养条件下,小菜蛾 AChE、CarE 和 GST 活力即受到显著抑制,AChE 是重要的神经递质水解酶,而 GST 是小菜蛾对甲胺磷抗性重要的解毒酶^[2],AChE 和 GST 被抑制将影响小菜蛾的正常生理状况及对杀虫剂的解毒代谢能力,高

温对小菜蛾 AChE、CarE 和 GST 活力的抑制将导致田间较低的用药剂量即可杀死小菜蛾,这也可造成夏季杀虫剂选择压力下降。

表 2 不同温度下饲养的抗性小菜蛾幼虫 AChE、CarE 和 GST 活性比较

Table 2 Comparison of the activities of acetylcholinesterase (AChE), carboxylesterase (CarE) and glutathione-S-transferase (GST) in *P. xylostella* larvae reared at the different temperatures

饲养温度 Raising-temperatur	AChE (n mol · mg ⁻¹ · min ⁻¹)		CarE (n mol · mg ⁻¹ · min ⁻¹)		GST (n mol · mg ⁻¹ · min ⁻¹)	
	酶活力±SE	比值	酶活力±SE	比值	酶活力±SE	比值
	Activity±SE	Ratio	Activity±SE	Ratio	Activity±SE	Ratio
35 C 2 d	3.62±0.02 b	0.71	107.58±9.27 b	0.72	85.56±7.78 b	0.77
25 C 2 d	5.11±0.48 a	1.00	149.32±16.28 a	1.00	111.91±5.19 a	1.00
5 C 4 d	4.89±0.30 a	0.96	121.34±11.51 ab	0.81	110.34±12.29 a	0.99
5 C 30 d	3.83±0.79 a	0.75	49.48±2.97 c	0.33	76.94±4.28 b	0.69

* 小菜蛾幼虫置于工气候箱中分别于 35 C、25 C、5 C 和 5 C 下饲养 2d、2d、4d 和 30d 后,取发育至 4 龄的幼虫用于酶活性测定。3 种饲养温度对酶活性影响的生化测定同时进行 *P. xylostella* larvae were reared in an environmental chamber at 35, 25, 5 and 5 C for 2, 2, 4 and 30 days, respectively, and then 4th instar larvae were used to determine the activity of AChE, CarE and GST; The biochemical analysis for the larvae with the different rearing temperatures was conducted simultaneously

表 3 不同温度下饲养的抗性小菜蛾幼虫 AChE 的 *Ki* 值测定结果

Table 3 Comparison of *Ki* of AChE in *P. xylostella* larvae reared at the different rearing temperatures

饲养温度 Raising-temperatur	甲胺磷 Methamidophos		克百威 Carbofuran	
	<i>Ki</i> ±SE (mol/L) ⁻¹ · min ⁻¹	比值 Ratio	<i>Ki</i> ±SE (mol/L) ⁻¹ · min ⁻¹	比值 Ratio
35 C 2 d	(2.20±0.29)×10 ² a	1.08	(1.35±0.08)×10 ⁴ a	1.07
25 C 2 d	(2.03±0.25)×10 ² a	1.00	(1.26±0.07)×10 ⁴ a	1.00
5 C 30 d	(2.07±0.12)×10 ² a	1.00	(1.19±0.13)×10 ⁴ a	0.95

* 小菜蛾分别在人工气候箱中于 35 C、25 C 和 5 C 下饲养 2d、2d 和 30d,取 4 龄小菜蛾幼虫于 25 C 下测定 AChE 的 *Ki* 值;3 种饲养温度对 AChE 的 *Ki* 值影响的生化测定同时进行 *P. xylostella* larvae were reared in an environmental chamber at 35, 25 and 5 C for 2, 2 and 30 days, respectively, and then 4th instar larvae were used to determine the *Ki* of AChE; The incubating temperatures of AChE and insecticides were 25 C. The biochemical analysis based on the effects of different rearing temperatures on *Ki* of AChE were conducted simultaneously

表 4 不同反应温度下 3 种杀虫剂对小菜蛾幼虫 AChE 的 *Ki* 值[(mol/L)⁻¹ · min⁻¹]测定结果

Table 4 Comparison of *Ki* [(mol/L)⁻¹ · min⁻¹] of AChE to dichlorvos, methamidophos and carbofuran at the different incubating temperatures in *P. xylostella* larvae collected from the field in Fuzhou

反应温度(C) Incubating temperatur	敌敌畏 Dichlorvos		甲胺磷 Methamidophos		克百威 Carbofuran	
	<i>Ki</i> ±SE	比值 Ratio	<i>Ki</i> ±SE	比值 Ratio	<i>Ki</i> ±SE	比值 Ratio
37	(8.69±0.60)×10 ³ b	1.60	(3.01±0.21)×10 ² b	1.33	(3.22±0.21)×10 ⁴ b	1.75
25	(5.45±0.19)×10 ³ a	1.00	(2.26±0.16)×10 ² a	1.00	(1.84±0.09)×10 ⁴ a	1.00
5	(4.59±0.13)×10 ³ a	0.84	(1.79±0.07)×10 ² a	0.79	(1.45±0.33)×10 ⁴ a	0.80

* 小菜蛾在人工气候箱中于 25 C 下饲养,取 4 龄小菜蛾幼虫测定 AChE 的 *Ki* 值,酶与杀虫剂的反应温度分别为 37 C、25 C 和 5 C *P. xylostella* larvae were reared in an environmental chamber at 25 C, and then 4th instar larvae were used to determine the *Ki* of AChE; The incubating temperatures of AChE and insecticides were 37 C, 25 C and 5 C, respectively

昆虫对有机磷的抗性与其最重要的靶标酶 AChE 敏感性(*Ki*)降低有关,这已在绝大多数已经进行研究的昆虫中得到证实^[16],福州地区田间小菜蛾对甲胺磷的抗性机制与 AChE 敏感性降低及解毒酶 GST 活力增高有关^[2,3],甲胺磷抗性小菜蛾脱离选择压力 8 代后,对甲胺磷敏感性(*LC*₅₀)降低 6.8 倍,同时 AChE 对甲胺磷、敌敌畏和克百威的敏感性(*Ki*)分别增高 3.6、13.9 和 3.5 倍,但抗性和敏感小菜蛾各虫态之间 AChE 活性及 *Ki* 值差异不显著^[3],本研究表 3 显示,在 AChE 与有机磷反应温度均为 25 C 的条件下,不同饲养温度对小菜蛾 AChE 的 *Ki* 值(对杀虫剂的敏感性)无显著影响,但由于田间一年四季气温变化很大,有机磷对小菜蛾 AChE 的抑制并不是发生在恒温条件下的,而表 4 的结果证实,在不同的反应温度条件下,有机磷对 AChE 的抑制作用差异很大,在高温下对小菜蛾 AChE 的抑制率(*Ki* 值)显著增高,因此,至少对有机磷和氨基甲酸酯类杀虫剂而言,在 6~8 月份高温期间较低剂量的杀虫剂即可导致小菜蛾死亡,这可导致田间杀虫剂选择压力下降,这可能也是导致 7 月份小菜蛾对甲胺磷和敌敌畏敏感性急剧升高的一个重要原因。此外,田间小菜蛾在 7 月份对有机磷敏感性急剧升高还可能与高温下虫体代谢速率升高(杀虫剂与靶标作用机率增大)及杀虫剂穿透率升高有关。

长时间低温的饲养条件可抑制小菜蛾 AChE 及解毒酶 CarE 和 GST 的活力,但短期低温(5℃,4d)对小菜蛾的 AChE、CarE 和 GST 活力影响不大(表 2),福州地区每年冬季一天中最低气温低于 5℃的时间仅几天,短期低温(5℃,4d)对小菜蛾的 AChE、CarE 和 GST 活力影响不大(表 2),总体上看,在福州地区冬季气温条件对与小菜蛾有机磷抗性有关的 AChE、CarE 和 GST 影响不大。生测结果显示,冬季小菜蛾对有机磷的抗性水平仍处较高水平。

本研究中杀虫剂毒力生物测定均在 25℃下进行,因此排除了测试温度对杀虫剂毒力影响的因素。昆虫抗性机制的形成与杀虫剂选择压力有关,这已成为人们的共识^[16],但本文的研究结果证实环境温度也会影响昆虫的抗性机制。夏季小菜蛾对有机磷杀虫剂抗性水平显著下降,究其原因,作者认为环境温度可通过影响小菜蛾虫口密度而影响田间用药动态(杀虫剂选择压力的季节性变化)、直接影响小菜蛾的生理和生态适合度^[13~15]并且影响与小菜蛾抗性有关的酶系(AChE、CarE 和 GST),而影响小菜蛾的抗性水平。在抗性治理中应考虑田间小菜蛾抗性水平的季节性变化而采取相应的用药对策。

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